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# Kepone-Induced Scoliosis and Its Histological Consequences in Fish

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16. ABSTRACT

Scoliosis in fish is caused by several diverse agents that possibly act on the central nervous system, neuromuscular junctions, or ionic metabolism. The organochlorine pesticide Kepone induces scoliosis in the sheepshead minnow. Some effects associated with Kepone-induced scoliosis in these fish are disruption of myotomal patterns, inter- and intramuscular hemorrhage, fractured centra of vertebrae, and death. The histological syndrome of Kepone poisoning in fish and the clinical syndrome in humans suggest that the nervous system is a primary target for Kepone and that scoliosis is a secondary effect of Kepone poisoning in fish.

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cent of the DNA is hybridized (Fig. 2), as was the case for RNA made in vivo (Fig. 1A). This result may be interpreted in two ways. (i) Failure to observe an increase in complexity of high abundance class mRNA may indicate that termination signals are obeyed on the frequently transcribed operons. This could happen in the absence of regulatory proteins as in the case with transcription in vitro of the rRNA genes (*18*). (ii) Polymerase molecules on the frequently transcribed genes do overrun the termination signals in vitro, but operons that are infrequently transcribed in the cell are not extensively interspersed with the frequently transcribed operons. In this case, if they were highly interspersed, transcripts from many of the less active sequences would have been increased in abundance and a more complex high abundance class would have been observed. Interspersion involving more than 6000 nucleotides cannot be ruled out since this is the maximal length of most of the in vitro transcripts.

Saturation values obtained with the nucleoid RNA show that about 73 percent of the total DNA was transcribed (Fig. 2). This means that during in vitro synthesis transcription is not exclusively asymmetric. Since the endogenous RNA polymerases did not usually transcribe beyond about 6000 nucleotides, an increase in complexity equal to 25 percent of the DNA suggests that there are frequent interspersions between sense and nonsense sequences on a given strand of DNA. Examples of such interspersions have been found for prokaryotic chromosomes (*1*, *19*). This interpretation depends on earlier findings that RNA chains are not initiated under the conditions of in vitro synthesis (*17*). The greater complexity of the nucleoid RNA is observed largely at high $C_0t$ (100 to 2000 mole · sec/liter). Thus, this sequence interspersion may be concentrated in infrequently transcribed regions of the DNA. However, if termination signals are only very rarely missed in vitro, transcripts of nonsense sequences adjacent to frequently transcribed operons would also be observed at high $C_0t$.

The inflection of the curve at $C_0t$ 100 (Fig. 1A) is not evident in Fig. 2. Lack of data on nucleoid RNA leaves unclear the question of whether this transition has been eliminated by increased gradation of copy frequency of the in vitro synthesized RNA.

In conclusion, we have observed maximal hybridization values of about 48 percent in various experiments with three preparations of RNA. Considering that certain regions of promotor and terminator sequences are not transcribed [for example, 1 to 2 percent of the lac operon is not transcribed (*20*)], a saturation value of 48 percent indicates that all of the genomic information is transcribed, if transcription is asymmetric.

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11. The region at minute 72 on the *E. coli* genetic map contains 24 known loci (*1*). Since $4.1 \times 10^4$ base pairs of DNA are transferred per minute, the average gene in this region of the chromosomes is $(4.1 \times 10^4)/24$ or $1.7 \times 10^3$ nucleotides in length. This could be an overestimate of average size if all the loci located at 72 minutes have not been identified, or if this region contains genes that are larger than average. An underestimate would result if the genes in this area are smaller than average, or if the same gene has been characterized phenotypically such that two or more loci actually represent the same gene. However, this approach may provide a better estimate than would the use of the average polypeptide length because such an average is largely determined by abundant class proteins, which may not be representative of the diverse, infrequent proteins. Furthermore, mRNA would be somewhat larger than reflected by respective proteins if untranslated sequences exist.
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## Kepone-Induced Scoliosis and Its Histological Consequences in Fish

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Abstract. *Scoliosis in fish is caused by several diverse agents that possibly act on the central nervous system, neuromuscular junctions, or ionic metabolism. The organochlorine pesticide Kepone induces scoliosis in the sheepshead minnow. Some effects associated with Kepone-induced scoliosis in these fish are disruption of myotomal patterns, inter- and intramuscular hemorrhage, fractured centra of vertebrae, and death. The histological syndrome of Kepone poisoning in fish and the clinical syndrome in humans suggest that the nervous system is a primary target for Kepone and that scoliosis is a secondary effect of Kepone poisoning in fish.*

We have found that exposure of sheepshead minnows (*Cyprinodon variegatus*) to a relatively low concentration of the organochlorine Kepone (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one) produces a syndrome in the fish in which scoliosis, resulting in severe spinal column injury, is one cardinal sign. Scoliosis, lateral curvature of the spine, has been reported to occur in several species of fish as a result of dietary deficiencies (*1*), organophosphate and carbamate poisoning (*2*), heavy metal exposure (*3*), and parasitic infections (*4*). This report is concerned with the severe histological effects of scoliosis in sheepshead minnows exposed to Kepone in the laboratory.

Hansen and co-workers (*5*) first observed scoliosis and blacktail (loss of neurologic control of melanocytes in the caudal region) as a syndrome of Kepone exposure in sheepshead minnows. They experimentally demonstrated that induction of scoliosis with Kepone was a dose-dependent, time-related phenomenon.

Scoliosis occurred in sheepshead minnows exposed 1 day to 24 μg of Kepone per liter, 2 days to 7.8 μg of Kepone per liter, 3 days to 1.9 μg of Kepone per liter, and 11 days to 0.8 μg of Kepone per liter. All tests were laboratory-controlled flowing seawater exposures.

We repeated exposures of sheepshead minnows to Kepone in the laboratory to obtain specimens exhibiting scoliosis for histological study. Twenty juvenile and ten adult sheepshead minnows were exposed for 17 days to flowing seawater containing 4 μg of Kepone per liter at 21° to 30°C and 23.5 to 29 parts per thousand salinity. Triethylene glycol was the solvent carrier for Kepone. A second equivalent group of control fish received only the solvent carrier chemical in flowing seawater.

All exposed fish exhibited signs of Kepone poisoning by day 10 of exposure. These fish demonstrated scoliosis, blacktail, loss of equilibrium, sporadic hyperkinesis, and tetanic convulsions. Living specimens with various degrees of scoliosis were selected from day 10 to day 17 of exposure for standard histological fixation and processing. Fish were fixed in Davidson's fixative; embedded in paraffin, and serially sectioned at 7 μm—some through a longitudinal, horizontal plane (Fig. 1, a and b) and others through a sagittal-parasagittal plane. Normal control fish were processed similarly. Selected fish with scoliosis and control fish were xeroradiographed for study of spinal column form.

Histological study revealed that fish with incipient scoliosis had precaudal trunk flexures resulting from long-term rigorous contractions of skeletal muscle in myotomes on one of either side of their bodies (Figs. 1a and 2). Xeroradiographs show that the major spinal column flexure occurs in the vicinity of, and involves, vertebrae 17 through 21 (cephalic to caudal count). We have counted 26 to 27 nonfused vertebrae in several normal specimens of the sheepshead minnow (Fig. 3). Normal muscle bundle patterns are broken and myotome boundaries (septa) are obscured by the abnormally contorted muscle bundles (Fig. 2).

In more advanced cases of scoliosis, hemorrhagic foci often occurred in affected myotomes, suggesting a rupture of minor vessels or capillaries possibly caused by severe contortion of muscle. The most striking effect observed, always in severely scoliotic fish, was the breaking of the centra of vertebrae at the epicenter of flexure in the spinal column (Figs. 4 and 5). This was accompanied by apparent osteoblastic repair tissue (Fig. 5, arrows) suggesting that the fractures occurred sometime prior to fixation of the fish for histology. Fish with fractured vertebrae also had the greatest displacement and disorientation of myotomal musculature (Fig. 5). Obstruction (pinching) of the dorsal aorta or caudal artery and severe imposition of displaced bone into the neural canal were further concomitant results of severe scoliosis (Fig. 6). Fish thus affected were paralyzed and probably would have died.

No histological lesions in the central nervous system that could have initiated tetany or paralysis were observed. We propose, however, that scoliosis is secondary to tetany and paralysis of trunk musculature initiated by an undetermined molecular neurological or neuromuscular dysfunction (or both), probably directly caused by Kepone. The appearance of blacktail (loss of neurologic control of caudal melanocyte patterns) preceding, during, and after scoliosis strengthens the possibility that the initiating lesion is neurologic (6). Another possibility is that systemic calcium metabolism may have been affected by Kepone. Evidence from living and fixed fish

Fig. 1. (a) Longitudinal histological section from the horizontal plane of normal control fish; section is at level of spinal column (×3.5). (b) Longitudinal horizontal plane section of fish with advanced scoliosis; the concave region in trunk (arrow) is location of flexure (×3.5). Fig. 2. Contorted muscle bundles (arrows) in myotome in concave region shown in Fig. 1b; the contorted musculature reflects the tetanic paroxysms that lead to scoliotic flexure of the trunk. Compare Figs. 2 and 4 (×160). Fig. 3. Xeroradiographs of normal sheepshead minnow (a) and sheepshead minnow with incipient scoliosis (b); the region of early spinal column flexure (arrows) shows obvious loss of vertebral periodicity. Fig. 4. Horizontal plane section from normal fish showing normal alignment of vertebral centra and normal myotomes in the region of trunk affected by Kepone-induced scoliosis in exposed fish (×160). Fig. 5. Fractured centra of vertebra in severely scoliotic fish exposed to 4 μg of Kepone per liter for 17 days; distortion of myotomes and foci of osteoblastic repair tissue at points of breaks in centrum walls is shown (arrows) (×64). Fig. 6. Horizontal section through the spinal cord of fish used in Fig. 5 with fractured vertebra; intrusion of disoriented bone (arrows) from neural arch of vertebra against lateral funiculi of spinal cord is shown (×64).

indicate that trunk muscles are unable to regain normal tonus and form (Fig. 2). Tetanic convulsions or chronic muscular rigor (or both) are associated with scoliosis and probably produce the fractured vertebrae of severely affected fish. The possible effects that Kepone may have on fish calcium metabolism, on the corpuscles of Stannius (because of their calcium-mediating role in some fishes), and on muscle contraction have not yet been evaluated.

The mechanism or mechanisms whereby different organochlorine compounds affect organisms are poorly understood. Human victims of Kepone poisoning have suffered tremors, nervousness (hyperkinesis), loss of memory, and slurred speech, among other effects (*7*). The human response syndrome suggests neurological lesions, some of which probably occur at higher nervous centers, as a result of Kepone poisoning. Tremors and other neurological-dependent responses in laboratory animals increased in severity with increasing Kepone concentration and duration of exposure (*7*). Hansen, *et al.* (*5*) observed the same correlation between concentration of Kepone, duration of exposure, and severity of scoliosis and related signs in fish. Our observations suggest that the severity of scoliotic effects in the sheepshead minnow is related to the duration of continuous exposure to a single low Kepone concentration (4 μg/liter). Much higher concentrations of Kepone (2 to 400 mg/kg per day) are required to elicit neuropathological, reproductive, and tissue effects in birds or mammals (*7*).

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## Limbic System Interrelations: Functional Division Among Hippocampal-Septal Connections

Abstract. *Neuronal activity was recorded simultaneously from hippocampus and medial or lateral septum during classical conditioning of the rabbit nictitating membrane response. Although similarities exist between hippocampal and lateral septal patterns of activity, medial septal unit discharges indicate a different role during learning.*

Interrelations between hippocampus and septum have been a major focus of neurophysiological and anatomical investigation (*1*). Recent evidence implicates the hippocampal-septal system in learning (*2*). Our laboratory has recently reported dramatic changes in neuronal activity in the hippocampal formation during classical conditioning of the nictitating membrane response of the rabbit (*3*). We now relate unit activity of the medial and lateral septal nuclei to hippocampal neuronal plasticity using this paradigm.

Methodological details have been described previously (*3*). Two microelectrodes per animal were permanently implanted, one in the dorsal hippocampus (CA1 or CA3) and one in either the medial or lateral septum. After 1 week of recovery, animals in the conditioning group were given 13 blocks of trials per day, with eight CS-UCS (*4*) paired trials and one CS-alone (1-khz, 85-db, 350-msec tone) test trial per block. The UCS was a 100-msec air puff to the cornea, onset 250 msec after CS onset. Animals were given one, sometimes two, days of conditioning. Control animals received 13 blocks of unpaired CS and UCS presentations per day, with eight CS-alone and eight UCS-alone presentations, for 16 unpaired trials per block. Data from 19 conditioning and 8 control animals are reported here.

Multiple-unit activity (*3*) was recorded simultaneously from hippocampus and septum during all phases of training. Recordings were subsequently band-pass filtered, with a pulse-height discriminator set to pass only the larger units. Unit analysis consisted of computing (i) the mean and standard deviation of cell discharges occurring 250 msec prior to CS onset (pre-CS period) and (ii) the mean number of spike events during equal intervals after CS onset (CS period) and UCS onset (UCS period). A standard score was computed for each block of trials for the CS period and the UCS period relative to the pre-CS period (*3*). Poststimulus histograms of the total number of neural responses (per 15-msec time bin) in all three periods were also constructed for each block. Behavioral analysis consisted of an analog-to-digital conversion of the amplitude-time curve of nictitating membrane movement for each trial. At the completion of training, animals were anesthetized, current was passed through each electrode, and placements were verified histologically after perfusion.

Analysis of lateral septal neuronal records ($N = 10$) revealed, in all but one case, the same pattern of unit activity seen in previously reported hippocampal results (*3*). Figure 1, A and B, shows the average nictitating membrane responses plus hippocampal and lateral septal poststimulus histograms from a typical animal at early and late phases of conditioning. Both hippocampal and lateral septal recordings show a rapid growth of unit activity in the UCS period early in training, long before behavioral conditioning (Fig. 1A). In addition, the pattern of unit firing, as represented by both poststimulus histograms, temporally precedes and parallels the amplitude-time

Table 1. Mean standard scores of unit activity in the CS period (CSP) and the UCS period (USP) computed from hippocampal (paired $N = 11$; unpaired $N = 5$), lateral septal (paired $N = 10$; unpaired $N = 4$), and medial septal (paired $N = 9$; unpaired $N = 4$) electrode site recordings during training trials.

| Block | Hippocampus | | Septum | | | |
|---|---|---|---|---|---|---|
| | | | Lateral | | Medial | |
| | CSP | USP | CSP | USP | CSP | USP |
| Paired | | | | | | |
| First | 1.27 | 6.91 | 1.47 | 5.44 | 4.19 | 7.78 |
| Last | 5.42 | 15.90 | 5.02 | 13.29 | 1.63 | 4.00 |
| Unpaired | | | | | | |
| First | 0.18 | 1.80 | 0.13 | 3.04 | 2.50 | 6.84 |
| Last | 0.33 | 3.22 | 1.34 | 1.81 | 0.29 | 5.40 |